# Trypan Blue Exclusion Protocol

The following procedure will enable you to accurately determine the cell viability.  Cell viability is calculated as the number of viable cells divided by the total number of cells within the grids on the hemacytometer.  If cells take up trypan blue, they are considered non-viable.

1. Determine the cell density of your cell line suspension using a hemacytometer.
2. Prepare a 0.4% solution of trypan blue in buffered isotonic salt solution, pH 7.2 to 7.3 (i.e., phosphate-buffered saline).
3. Add 0.1 mL of trypan blue stock solution to 1 mL of cells.
4. Load a hemacytometer and examine immediately under a microscope at low magnification.
5. Count the number of blue staining cells and the number of total cells.  Cell viability should be at least 95% for healthy log-phase cultures.

% viable cells = [1.00 – (Number of blue cells ÷ Number of total cells)] × 100  
  
To calculate the number of viable cells per mL of culture, use the formula below.  Remember to correct for the dilution factor.  
  
Number of viable cells × 104 × 1.1 = cells/mL culture

Taken from: <http://www.lifetechnologies.com/us/en/home/references/gibco-cell-culture-basics/cell-culture-protocols/trypan-blue-exclusion.html>